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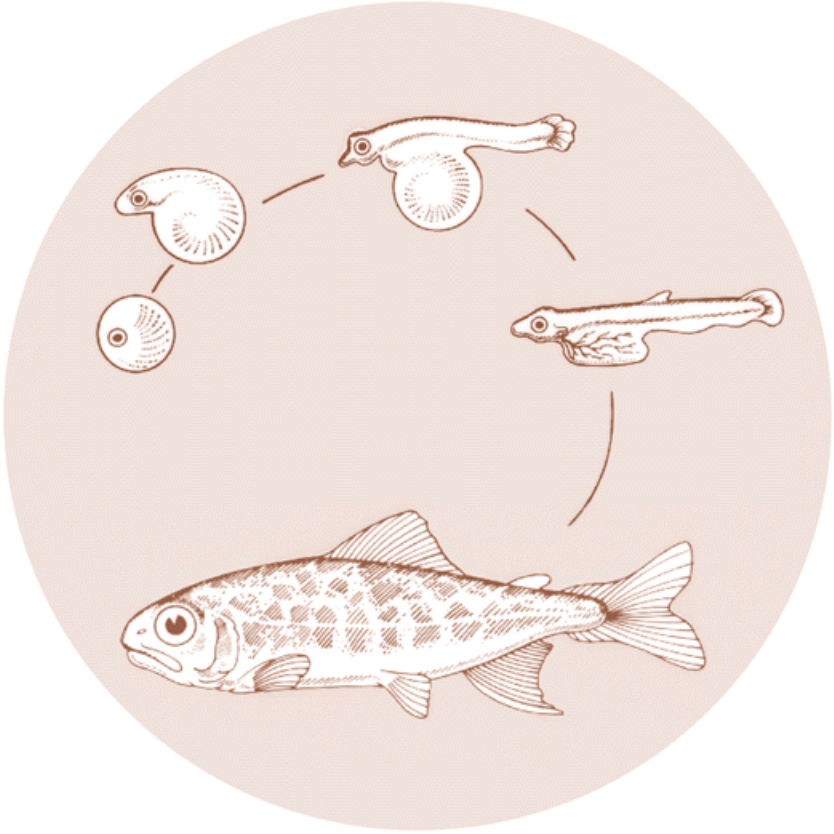
# RESEARCH TO IDENTIFY EFFECTIVE ANTIFUNGAL AGENTS

Annual Report 1992



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# RESEARCH TO IDENTIFY EFFECTIVE ANTIFUNGAL AGENTS

**ANNUAL REPORT 1992**

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## Abstract

The special permit to use malachite green as a fungicide in culture of endangered and restoration species of fish was canceled on August 27, 1991; the need for a replacement fungicide has intensified. Selected candidate chemicals were tested on cultured fungus for growth inhibition and on infected eggs of rainbow trout to evaluate their antifungal activity. The candidate with the best potential was further tested for antifungal activity on adult spring chinook salmon. Candidate chemicals were Amorolfine, Clotrimazole, Fenpropidin, Fenpropimorph, glutaraldehyde, iodine, hydrogen peroxide, sodium chloride, potassium permanganate, and VigorOx and reference fungicides were malachite green and formalin. Since infection rates in these tests were high at 20% or greater, higher concentrations of malachite green (5.0 ppm) and formalin (1,667 ppm) were required to demonstrate fungicidal activity. The most effective compounds other than reference chemicals were sodium chloride at 30,000 ppm, glutaraldehyde at 200 ppm, and VigorOx at 200 ppm. Iodine and potassium permanganate at 100 ppm were fungistatic only at this high level of infection. Registrations for Amorolfine, glutaraldehyde, and VigorOx are nonexistent as therapeutants; their development would require further studies and negotiations with regulatory agents. Results of these studies suggest that Formalin at 1,667 ppm and sodium chloride at 30,000 ppm are superior to the other compounds tested. Tests on adult spring chinook salmon indicated that glutaraldehyde at 6 or 31 ppm was as or more effective than formalin at 167 ppm as a fungicide. Glutaraldehyde was toxic at 62 ppm, but not at 6 or 31 ppm.

## Introduction

Aquatic fungi (Saprolegniales) are ubiquitous in natural water supplies of fish hatcheries often causing serious disease problems. Malachite green is effective in control of fungus on fish and fish eggs, but due to suspected teratogenicity (Meyer and Jorgenson 1984) its use was limited to the treatment of non-food fish (i.e., eggs or adult salmon held for spawning) under an Investigational New Animal Drug Application (INAD) held by the U.S. Fish and Wildlife Service. That INAD was canceled on August 27, 1991 and uses were to be discontinued within 45 days. Heretofore special exemptions are required by the FDA for any uses. Presently, there is one registered aquatic fungicide, formalin, but it is not completely satisfactory in control of fungus on fish or their eggs. Consequently, the search for safe and effective aquatic fungicides must continue.

Bailey (1984) and Bailey and Jeffrey (1989) reported results of tests with over 200 compounds that were chosen for fungicidal activity. More than half were found to be unsuitable as aquatic fungicides in preliminary tests because of their lack of activity against fungi, toxicity to fish or their eggs, insolubility in water, or potential carcinogenicity. However, several of the better candidates showed potential for control of fungus on fish eggs and were chosen for further evaluation.

This study is a continuation of "Research to Identify Effective Antifungal Agents" sponsored by Bonneville Power Administration (Schreck et al. 1990 and Schreck et al. 1991). The objectives of the present study were to select and evaluate up to 10 candidate fungicides. Evaluations involve laboratory studies on efficacy of candidate compounds on cultured Saprolegniales, on eggs of rainbow trout and chinook salmon that were

previously infected with the fungus, and hatchery studies on adult spring chinook salmon.

## Goal I. Eggs and Juveniles

### Materials and Methods

#### In Vitro Tests

Pure strains of aquatic fungi were obtained from the American Type Culture Collection (ATCC). Saprolegnia hypogyna (ATCC 28275) was used for both range finding and minimum inhibitory concentration tests. Test procedures used were those developed by Bailey (1983a,b). The method involved an in vitro screening technique modified from that of Golden and Oster (1947) and a minimum inhibitory concentration determination based on the percent inhibition of growth in diameter of colonies (Bailey and Jeffrey 1989).

Chemicals in solid or liquid form were weighed or measured and dissolved in aqueous solutions; concentrations were corrected for purity but not for specific gravity. For example, malachite green was prepared from a 50% active solution; twice the volume of stock was added to the dilution media to account for purity. Solid samples included Amorolfine, Clotrimazole, sodium chloride, and potassium permanganate. For continuity, concentrations are reported in ppm for solid and liquid chemicals in these tests.

*Range Finding.* Standard petri dishes were filled with 20 ml of corn meal agar and inoculated with agar plugs augmented with fungi (5 mm in diameter). The fungi was allowed to incubate at 20°C for approximately 96 hours. Agar plugs were removed from the edge of the colonies with a #l cork

borer. Stock solutions of chemicals were prepared to achieve final concentrations of 1, 10, and 100 ppm. The depressions of Coors porcelain spot plates were filled with three replicates of each of the candidate chemicals and the solvent, positive, and negative controls. Agar plugs were then added to the depressions of the spot plates for exposures of 15 and 60 min. Agar plugs were removed from the spot plates, rinsed three times with sterile distilled water, and placed on tri-petri dishes containing 30 ml of corn meal agar. Cultures were incubated in continuous light inside an environmental control chamber maintained at 20°C (+ 2°C). The plates were examined for mycelial growth after 48, 96, and 168 hours of incubation.

*Minimum Inhibitory Concentration.* Inoculations, incubation, and stock solution preparation were as stated above. Agar plugs were removed from the edge of the fungal colonies as previously described and exposed in triplicate to five delineative concentrations between 0.1 and 1.0 ppm, 1.0 and 10.0 ppm, or 10.0 and 100.0 ppm, or at higher levels, depending on the activity observed in the range-finding test.

The agar plugs were exposed to the test chemicals in triplicate for 15 or 60 min. They were rinsed with sterile distilled water and placed on standard petri dishes containing 10 ml of corn meal agar. Cultures were incubated in a lighted environmental control chamber at 20°C for 48 hours, and the colony diameters were measured with a vernier caliper.

In Vivo Tests

Green eggs from Trout Lodge (Sumner, Washington) were placed in Heath incubating trays (500 eggs per tray) with the use of a modified egg counting board. Characteristics of the well water used for incubation was a total

hardness of 138 ppm as $CaCO_3$, alkalinity of 105 ppm as $CaCO_3$, pH of 8.0, and temperature of 12°C. Concentrations of dissolved oxygen remained at 9.0 ppm or above during the exposures. Water flow was about 1 L/min during incubation and treatments. During infection water flow was discontinued for 2 h periods in morning and afternoon to promote infection of the eggs. The eggs were confined within a 6-inch diameter acrylic ring that was 1 inch in height and attached with silicone to the screen of each incubator tray. Two trays of 500 eggs each were used as replicates for each treatment level. Eggs were inoculated with 12 S. ferax infected hemp seeds suspended by a tea ball in the upper tray of each replicate treatment for exposures of approximately 7 days or until the initial infection rate was about 20% or greater. Prior to exposure infection rates were equalized by exchanging infected eggs in trays with a high infection rate with uninfected eggs in trays with a low infection rate. Eggs were exposed to the fungicides for 15 or 60 min.

The chemicals were delivered to the water inflow of a separate mixing tray above the egg hatching trays with the use of a peristaltic pump to achieve specific desired concentrations. The mixing tray contained a maze of baffles to ensure complete mixing. Concentrations were calculated on the basis of amount of material added to a specific volume of water flow. Treatments were administered three times weekly for a period of 2 weeks or until eggs began hatching. Mortality and fungal infection were assessed prior to the first treatment (pretreatment), after the last treatment (post-treatment), and after the eggs hatched (post-hatch). Infection rates (% increase) were calculated by subtracting pretreatment infection rates from post-treatment rates. The percent hatch was corrected for initial

mortality by subtracting the pretreatment mortality from the total number hatched according to the following formula.

$$\text{Percent hatch} = \frac{\text{number hatched}}{\text{total} - \text{initial morts}} \times 100$$

Toxicity Testing

Toxicity of the candidate fungicides was performed in the egg incubators simultaneously with the efficacy treatments. The setup was the same as stated above for the in vivo testing; however, the eggs were uninfected. The dilution series was generally based on a use pattern of 1, 3, and 5X. The 1X concentration was the concentration we felt would be effective for control of fungus. Mortality observations were taken daily and egg hatching success was recorded at the end of each test. Margins of safety for each chemical were established by dividing the toxic concentration by the effective concentration for respective exposure times.

Concurrent Exposure

Past results have shown seasonal variability in the infection rates and effectiveness of some antifungal agents. During a three month period concurrent exposures of the better candidates were undertaken to minimize these effects. These exposures were for 60 minutes and the antifungal candidates were tested on the same group of eggs. Each month 2,000 eggs were exposed in each treatment with two replicates of 1,000. The candidates chosen for these exposures were Amorolfine, glutaraldehyde, iodine, potassium permanganate, sodium chloride, and VigorOx along with formalin and malachite green as reference compounds.

## Results

Ten chemicals were evaluated for antifungal activity on cultured fungus *(S. hypogyna)* on the basis of their minimum effective concentration and safety (Table 1). All of the compounds were antifungal, especially at the 60 minute exposure period, but high levels were required of some of the candidates. Malachite green was used as a reference chemical because of its known antifungal activity. However, we found that 5.0 ppm in vivo were required to give satisfactory results. The higher treatment rate for malachite green is perhaps related to higher infection rates (usually 20% or greater) in the infected eggs whereas they are treated at lower infection rates in hatchery situations.

### Fenpropidin and Fenpropimorph

Fenpropidin and Fenpropimorph are structurally similar to the morpholine derivative called Amorolfine which demonstrated antifungal activity previously. These chemicals possess remarkable antifungal activity against a broad spectrum of fungus that are pathogenic to plants, animals, and humans. Fenpropidin was somewhat effective at 300 ppm in 60 minutes exposure; the infection rate decreased but the hatch rate did not show improvement (Table 2). Toxicity was noticeable at 300 ppm and evident at 500 ppm in the 60 minute exposures. Fenpropimorph was evaluated on rainbow trout eggs that were highly resistant to infection; exposure had to be started with low infection rates (Table 3). The effective exposure was at 300 ppm for 60 minutes and toxicity was not apparent at 500 ppm. Formulations of these materials were not soluble in water and precipitation

occurred even when ethanol was used as a carrier. Neither of these analogs was as effective as Amorolfine and will not be tested further.

Glutaraldehyde

Glutaraldehyde, a five-carbon dialdehyde with some commercial and clinical uses, demonstrated antifungal activity at levels much lower than formaldehyde. Concentrations of 50, 100, and 200 ppm were effective for decreasing infection rates and increasing hatching rates (Table 3). Antifungal activity was especially noticeable in the 60 minute exposures. Toxicity was apparent in the 60-minute exposures, moderately at 300 ppm and extensively at 500 ppm. In addition to mortalities, the treatments at these high levels caused a delay in hatching for up to one week. Glutaraldehyde demonstrated desirable antifungal activity and further testing and evaluations are warranted.

Potassium permanganate

Potassium permanganate has been reported to be effective for control of fungal infections on trout eggs. The EPA has allowed its use as an oxidant and a detoxifier in water treatment processes. Treatment levels of 2 ppm and effluent levels of 0.05 ppm were considered safe. Exposure concentrations of 25 to 100 ppm did not effectively control the fungal infections and the hatch rates were not improved. Toxicity was apparent in 60 minute exposures at 150 and 250 ppm. The higher levels of potassium permanganate caused heavy staining on the eggs which complicated mortality evaluations. Also, the hatching was delayed by a few days at the higher treatment rates. The use of potassium permanganate as a therapeutant in

fish culture would be regulated by the FDA and some kind of registration would be required. Potassium permanganate is not considered to be a potential antifungal agent at this time.

VigorOx

VigorOx is an effective sanitizing agent that is formulated to contain 5% peracetic acid and 20% hydrogen peroxide. It is approved by the EPA and accepted as safe by the FDA as a sanitizer for food surfaces. This non-corrosive, non-staining, oxidant provides advantages over other oxidants. Treatments of rainbow trout eggs for 60 minutes of exposure at 100 ppm was effective for decreasing infection rate and improving the hatch rate (Table 6). Toxicity was apparent at 250 ppm in the 60 minute exposure. Additional testing is warranted to further evaluate the potential of VigorOx for antifungal activity.

Concurrent exposures

Candidate antifungal agents were chosen from those tested the past two years for further evaluation. They were Amorolfine, glutaraldehyde, iodine, potassium permanganate, sodium chloride, and VigorOx in addition to reference compounds formalin and malachite green (Table 7). In the first trial, the 1,667 ppm concentration of formalin was considerably more effective than 250 ppm for decreasing the infection rate (-3.6%) and improving the hatch rate (87% vs 51%). In fact, the high treatment of formalin produced a better hatch rate than any of the candidate antifungal agents or malachite green. The next most effective treatment was the 30,000 ppm of sodium chloride. The increase in fungal infection was only 1.8% and

the hatch was 78.2%. All other candidates were less effective at rates tested, but there were some positive effects on infection and hatching rates.

Iodine exposures to 100 ppm for 60 minutes were extremely high and regulatory agents would not allow those levels without verification of safety. The FDA has concluded that povidone iodine compounds were low in regulatory priority when used at 100 ppm for 10 minutes as egg disinfectants after water hardening. Consequently, these iodine compounds can be used after water hardening but there is no formal approval for those uses and exposures. Iodine will not be evaluated further in these in vivo exposures, but it should be evaluated for efficacy at hatcheries.

Potassium permanganate exposures at 100 ppm for 60 minutes resulted in poor hatch, stained the eggs, and caused a delay in hatching. Potassium permanganate will not be evaluated further in this program.

The 1.0 ppm of malachite green also was not as effective as formalin or sodium chloride. Additional evaluations were done at higher treatment levels.

Subsequent exposures were done at identical concentration for two monthly trials. The results of duplicated exposure for the two separate trials are reported as the mean and standard deviation (Table 8).

The reference fungicide, malachite green, was tested at 5.0 ppm. This exposure level was necessary to be effective at the high infection rate of 25%. This treatment was effective for decreasing the infection in non-infected eggs and increased the hatch rate substantially over the negative control group of eggs. Although the prescribed treatment rate for malachite

green in fish culture is 1.0 ppm, that level would not be effective for treating high levels of fungal infection.

Formalin at 1,667 ppm was the most effective treatment on infected eggs. Infection rates were negative values which suggests that some of the infected eggs survived to develop and hatch. Hatch rates were highest among any of the candidates and malachite green. The low treatment level of 250 ppm was ineffective for treating this high rate of fungal infection.

Amorolfine was the most effective antifungal agent among three morpholine derivatives. In these trials it was ineffective for decreasing infection rates and the resulting hatch rates were not impressive even though they were better than the negative control. The manufacturer of Amorolfine has indicated it is no longer interested in the development of that material as an antifungal agent; the manufacturer's policy is that drugs for human use are not used for animal or crop care. Therefore, this material will not be evaluated further.

Glutaraldehyde at 200 ppm provided effective fungicidal activity; the increase in infection was only about 16% and the hatch rate about 36%. The literature suggests that high concentrations of glutaraldehyde may be mutagenic; additional information on safety is required to evaluate the potential of this material. Inquiries to FDA agents on registration requirements have confirmed the need to develop additional toxicology and teratology information. Glutaraldehyde continues to be a candidate antifungal compound for treatment of eggs.

Sodium chloride or mixtures of sodium and calcium chloride have been suggested and reported as a safe, efficacious, and economical treatment for saprolegniosis in salmonid incubation and rearing. Taylor and Bayle (1979)

reported that daily treatments of 2-3 hours with sea water were effective for control of *Saprolegnia declina* on eggs of pink salmon *(Oncorhynchus gorbuscha)*. In our studies 30,000 ppm or 3% were effective for decreasing infection rates and increasing hatch rates (Table 8). Salt remains a candidate fungicide, however, the large quantities required complicate the logistics of use and the disposal of effluent.

VigorOx at 200 ppm was not particularly effective for decreasing infection rate or improving the hatch rate. Additional testing of the components of peracetic acid and hydrogen peroxide are necessary to fully evaluate this oxidant as an antifungal agent.

## Goal II. Adults

### Materials and Methods

Based on results from Goal I, glutaraldehyde was selected as the candidate compound for testing on adult spring chinook salmon. Glutaraldehyde (25% solution) was purchased from VWR Corporation (Seattle, WA). Preliminary toxicity tests were performed on adult rainbow trout during final maturation to narrow the choices of exposure concentrations for spring chinook salmon. Groups (n=5) of adult rainbow trout were placed in 3-foot circulars and exposed to 62, 6.2, and 0.62 ppm glutaraldehyde every other day for one week.

On 12 and 61 June 1992, 124 and 78 adult spring chinook salmon, respectively, were delivered to Smith Farm Experimental Hatchery (Corvallis, OR) from Oregon Department of Fish and Wildlife's Dexter Holding Facility (Willamette River) and distributed among eight 10-foot circular tanks (flow

= 45 L/min; water temperature = 13-14°C). On 15, 17, and 19 June, all tanks were treated with formalin at 167 ppm as a prophylaxis. Any mortalities that occurred during this period were attributed to transportation stress. On 23 June, replicate tanks were treated in the following manner: controls (no treatment), formalin (167 ppm; equivalent to 62 ppm formaldehyde), glutaraldehyde (62 ppm) and glutaraldehyde (6.2 ppm). Treatment consisted of adding the appropriate volume of stock chemical to a bucket, diluting to 20 L with water, and then adding the contents to the tank at the inflow over 1 min. All adults received erythromycin and oxytetracycline before transport to the facility. Because of the toxic reaction to the highest concentration of glutaraldehyde, treatments were modified on 26 June as follows: formalin and glutaraldehyde (6.2 ppm) were maintained as before, controls were changed to glutaraldehyde at 31 ppm, and glutaraldehyde at 62 ppm was changed to 15 ppm (once per week). Although this change resulted in the loss of the unexposed control group (positive control), the efficacy of glutaraldehyde can still be compared to that of formalin (negative control). The tanks were checked daily for mortalities and any mortalities that did occur were scored for the presence of fungus on the body or gills, abrasions on the body, and obvious abnormalities in the organs. Gender and maturational status were also noted. Treatments were discontinued after the initial detection of ovulation on 10 September 1992.

## Results

Mature rainbow trout showed no toxic reaction to glutaraldehyde exposure at 62, 6.2, or 0.62 ppm. Therefore, 62 and 6.2 ppm glutaraldehyde were selected as exposure levels for adult spring chinook salmon.

Mortalities began within 8 hours of the initial exposure to 62 ppm glutaraldehyde and by 48 hours had reached 64 an 50%, respectively, in the two replicates (Table 9). No other treatments, including the lower glutaraldehyde exposure, suffered any mortalities during this period. One control fish and one survivor from the 62 ppm glutaraldehyde group were held separately and exposed to 31 ppm glutaraldehyde. No toxic reaction occurred and triweekly treatments commenced on 26 June as follows formalin at 167 ppm and glutaraldehyde at 31 ppm, 6.2 ppm, and 15 ppm (the latter at once per week on the survivors of the 62 ppm glutaraldehyde group).

In all treatments combined, 33 of 154 (21.4%) fish died between 26 June and 10 September, which marked the initial detection of ovulation. Only 7 of these mortalities had associate fungal infections. Total mortality ranged from 22.7 to 52.2% i formalin treatments compared with 4.5 to 8.7% in glutaraldehyde exposures at 31 ppm and 4.3 to 30.4% in glutaraldehyde exposures at 6.2 ppm (Table 10). Most mortalities from all treatments had no obvious signs of infection or tissue damage; however, many had pale, mottled livers. Final maturation was completed by 46 females and most of the males, and resulted in the production of about 192,000 eggs.

## Discussion

Willoughby and Robert (1992) reported that 0.25 ppm of malachite green oxalate killed zoospores an zoospore cysts fo *Saprolegnia parasitica* in the water column and that exposure for 15 minutes should contro fungal growth and protect the fish. In reality most fish culturists treat with malachite green at 1 ppm or higher for exposures of up t 1 hour. However, control of fungus on infected eggs requires higher concentrations, especially when

infection rates are at 20% or greater as in our experiments. The 5.0 ppm applications for 1 hour was fungicidal; whereas, the 1.0 ppm treatment is probably fungistatic. The higher levels required for antifungal activity on eggs are perhaps too excessive for use on fish.

Results of in vivo testing suggested that Amorolfine, glutaraldehyde, iodine, potassium permanganate, sodium chloride, and VigorOx showed potential for control of fungus on infected eggs. In the first concurrent exposure of infected eggs to antifungal agents (Table 7), the concentrations of malachite green, Amorolfine, glutaraldehyde, and VigorOx were inadequate to demonstrate desirable antifungal activity. The concentrations of iodine and potassium permanganate were as high as the eggs would tolerate, yet neither compound produced satisfactory antifungal activity. However, both compounds have been reported to be useful for treating or preventing fungal infections in cultured eggs. Perhaps either compound (possibly at lower concentrations) would be useful for treating eggs that had a lower infection rate. Iodine treatment of salmonid egg surfaces has been recognized as an effective means to prevent the spread of disease (Piper, et.al. 1982). The 100 ppm iodine for up to 60 minutes exposure was demonstrated to be safe and effective for water hardening salmonid eggs (Chapman and Roger 1992), however, they reported significant losses of the iodine concentration in treatment solutions to the eggs. Formalin at 1,667 ppm and sodium chloride at 30,000 ppm were the most effective compounds for control of fungal infection and increasing the hatch rate.

The remaining candidate antifungal agents, two levels of formalin, and 5.0 ppm of malachite green were tested simultaneously for two months. Results of these exposures (Table 8) suggest that the high level of

malachite green was antifungal for infection rates of about 23%. Formalin at 1,667 ppm was the most effective treatment; the infection decreased and the hatch rate was 73%. Sodium chloride was more antifungal than the remaining candidates and those treatments resulted in a better hatch. Amorolfine, glutaraldehyde, and VigorOx treatments produced a better hatch rate than the negative control groups, but improvement was only moderate.

Adult spring chinook salmon were more sensitive to the toxic effects of glutaraldehyde than adult rainbow trout; nevertheless, exposures to lower levels of glutaraldehyde showed promising results. The level of mortality suffered in glutaraldehyde-exposed salmon was lower than that in formalin-treated fish in most of the replicates. In addition, the number of mortalities with associated fungal infections was very small in all glutaraldehyde treatments and in the formalin treatment. In 1991, total mortalities and mortalities associated with fungal infection in untreated adult spring chinook salmon were 90.2 and 84.8%, respectively (Schreck et al. 1991). Because our methodology calls for allowing natural fungal infection of adult salmon, it could be argued that the low mortalities suffered by glutaraldehyde- and formalin-treated fish this year may have resulted from low incidence of fungi on fish transported to our facility this year. However, this is unlikely because once treatments were suspended at the onset of spawning, fungal infections became noticeable in the fish and 9 of the 27 mortalities that occurred between 10 September and 2 October had advanced fungal infections.

Glutaraldehyde offers promise as an effective antifungal agent for use on adult spring chinook salmon barring major problems with registration. The lowest exposure level tested (6.2 ppm) was effective at controlling

fungal infection and represents a lo-fold lower exposure level than that of formaldehyde, the active component of formalin. Lower exposure levels may also be effective and determination of minimum effective exposure levels should be pursued.

Registration requirements are rigid, expensive, and time consuming for compounds that have no other registered uses or for those that have not been tested for mammalian safety. Amorolfine, glutaraldehyde, an VigorOx have no existing registration by FDA for therapeutic uses. Their development would require further negotiations with regulatory agencies. At this time, none seem to comparehwit formalin or sodium chloride as an antifungal agent on salmonid eggs; however, glutaraldehyde seems to compare favorably with formalin for treatment of adults and carries the potential advantage of significantly lower exposure levels. Sodium chloride has been recognized as a safe treatment on fish and their eggs for disease and stress. Therefore, sodium chloride must be considered as an effective antifungal agent. However, the high concentrations required are logistically difficult for hatchery managers. If sodium chloride is to be used in fish culture, procedures will be required to implement administration and effluent treatment.

## Literature Cited

Bailey, T. A. 1983a. Method for in vitro screening of aquatic fungicides. Journal of Fish Disease 6:91-100.

Bailey, T. A. 1983b. Screening fungicides for use in fish culture: evaluation of the agar plug transfer, cellophane transfer, and agar dilution methods. The Progressive Fish-Culturis 45:24-27.

Bailey, T. A. 1984. Effects of twenty-five compounds on four species of aquatic fungi *(Saprolegniales)* pathogenic to fish. Aquaculture 38:97-104.

Bailey, T. A., and S. M. Jeffrey. 1989. Evaluation of 215 candidate fungicides for use in fish culture. U.S. Fish and Wildlife Service, Investigations in Fish Control 99. 9 pp.

Chapman, P. F. and R. W. Rogers. 1992. Decline in iodine concentrations of iodophor during water hardening o salmonid eggs and methods to reduce this effect. The Progressive Fish-Culturis 54:81-87.

Golden, J. J., and K. A. Oster. 1947. Evaluation of a fungicidal laboratory test method. Journal of American Pharmaceutical Association 36:350-362.

Meyer, F. P., and T. A. Jorgenson. 1984. Teratological and other effects of malachite green on development in rabbits and rainbow trout. Transactions of the American Fisheries Societ 112:818-824.

Piper, R. G., I. B McElwain, L. E Orme, J. P. McCraren, L. G. Fowler, and 3. R. Leonard. 1982. Fish hatchery management. U.S. Fish and Wildlife Service, Washington, D.C.

Polak, A., and M. Zaug. 1990. Amorolfine. Pages 505-537 in Handbook of Experimental Pharmacology (J. F. Ryley, editor). Vol. 96, Chapter 21.

Chemotherapy of fungal diseases. Springer-Verlag Berlin, Heidelberg, New York, Paris, Tokyo, Hong Kong, Barcelona.

Schreck, C., M. Fitzpatrick, L. L. Marking, J. J. Rach, and S. M. Jeffrey. 1990. Research to identify effective antifungal agents. Annual report 1990. Bonneville Power Administration, Portland, Oregon. 21 pp.

Schreck, C., M. S. Fitzpatrick, R. Gariss, L. L. Marking, J. J. Rach, and T. M. Semann. 1991. Research to identify effective antifungal agents. Annual report 1991. Bonneville Power Administration, Portland, Oregon. 31 PP.

Taylor, S. Gd an J. E. Bailey. 1979. Saprolegnia: Control of fungus on incubating eggs of pink salmon by treating with sea water. The Progressive Fish-Culturist, 41:181-183.

Willoughby, L. G. and R J. Roberts 1992. Towards strategic use of fungicides against *Saprolegnia* parasitica in salmonid fish hatcheries. Journal of Fish Diseases, 15:1-13.

**Table 1.** **Effectiveness (ppm) of candidate fungicides on cultured fungus (Saprolesnia hypogyna) and on eyed eggs of rainbow trout infected with fungus (S. ferax) in terms of MIC (minimum inhibitory concentration), MEC (minimum effective concentration), MSC (minimum safe concentration), and safety index.**

| Chemical Name | Exposure time (min) | In Vitro Range | In Vitro MIC | In vivo MEC | Toxicity (MSC) eyed eggs | Safety index eyed eggs |
|---|---|---|---|---|---|---|
| **Amorolfine** | **15** | >lOO | >300 | 300 | >500 | 1.7 |
| | 60 | >10<100 | >50 | 100 | >500 | 5.0 |
| **Clotrimazole** | 15 | >lO<lOO | >10<30 | - - | - - | - - |
| | 60 | >10<100 | ≤10 | - - | - - | - - |
| **Fenpropidin** | 15 | >lOO | >300<500 | 300 | >500 | >1.7 |
| | 60 | >lOO | >200<300 | 100 | (300 | <3.0 |
| **Fenpropimorph** | 15 | >lOO | ≥500 | 500 | >500 | >l.O |
| | 60 | >lOO | ≤100 | 300 | >500 | >1.7 |
| **Formalin** | 15 | >lOO | t300 | 1,667 | 5,000 | 3.0 |
| | 60 | >lOO | <300 | 1,000 | 1,667 | 1.7 |
| **Glutaraldehyde** | 15 | >lO<lOO | >50<75 | 200 | >500 | >2.5 |
| | 60 | >lO<lOO | >25<50 | 50 | <300 | <6.0 |
| **Iodine** | 15 | >10<100 | >70<100 | 50 | <500 | tlO.O |
| | 60 | >lO<lOO | >50<70 | 50 | (300 | t6.0 |
| **Malachite Green** | 15 | >l<lO | >1<3 | 1.0 | ≥5 | ≥5.0 |
| | 60 | >l<lO | >1<3 | 1.0 | ≥5 | ≥5.0 |
| **Hydrogen Peroxide** | 15 | >lO<lOO | >lOO | - - | - - | - - |
| | 60 | >lO<lOO | >lOO | - - | - - | - - |
| **Sodium Chloride** | 15 | >l,OOO | >50,000 | 50,000 | >50,000 | >l.O |
| | 60 | >l,OOO | >30,000 | 30,000 | >50,000 | >1.7 |
| $KMnO_4$ | 15 | >lO<lOO | >50<100 | 50 | >250 | >5.0 |
| | 60 | >lO<lOO | >50 | 25 | <150 | (6.0 |
| **VigorOx** | 15 | >lO<lOO | >20<60 | 100 | >250 | >2.5 |
| | 60 | >lO<lOO | <20 | 100 | <250 | (2.5 |

Table 2. Effectiveness of Fenpropidin on infected eggs of rainbow trout and toxicity of 1, 3, and 5X concentrations to uninfected eggs at 12°C.

| Treatment (ppm) | Exposure (min.) | Infecti on (%) Initial | Increase | Hatch (%) |
|---|---|---|---|---|
| **Efficacy** | | | | |
| (-) Control | - - | 0.0 | 59.6 | 27.9 |
| (t) Control | - - | 15.6 | 53.5 | 30.1 |
| 100 | 15 | 39.7 | 36.0 | 32.6 |
| 100 | 60 | 22.6 | 48.3 | 50.9 |
| 300 | 15 | 25.9 | 43.9 | 56.8 |
| 300 | 60 | 33.5 | 10.5 | 33.8 |
| 500 | 15 | 34.8 | 31.7 | 43.5 |
| 500 | 60 | 28.3 | 14.0 | 9.1 |
| **Toxicity** | | | | |
| (-) Control | - - | - - | - - | 83.9 |
| (t) Control | - - | - - | - - | 89.1 |
| 100 | 15 | - - | - - | 86.3 |
| 100 | 60 | - - | - - | 78.6 |
| 300 | 15 | - - | - - | 85.2 |
| 300 | 60 | - - | - - | 63.7 |
| 500 | 15 | - - | - - | 87.4 |
| 500 | 60 | - - | - - | 5.0 |

**Table 3. Effectiveness of Fenpropimorph on infected eggs of rainbow trout and toxicity of 1, 3, and 5X concentrations to uninfected eggs at 12°C.**

| Treatment (ppm) | Exposure (min.) | Infection (%) Initial | Increase | Hatch (%) |
|---|---|---|---|---|
| **Efficacy** | | | | |
| (-) Control | - - | 0.5 | 3.3 | 92.2 |
| (t) Control | - - | 0.0 | 1.1 | 94.0 |
| 100 | 15 | 13.2 | 3.7 | 85.6 |
| 100 | 60 | 14.6 | 3.5 | 91.4 |
| 300 | 15 | 16.7 | 3.4 | 89.6 |
| 300 | 60 | 19.1 | 1.0 | 90.3 |
| 500 | 15 | 14.8 | 2.0 | 91.2 |
| 500 | 60 | 13.4 | 0.5 | 91.9 |
| **Toxicity** | | | | |
| (-) Control | - - | - - | - - | 98.0 |
| (t) Control | - - | - - | - - | 97.5 |
| 100 | 15 | - - | - - | 97.5 |
| 100 | 60 | - - | - - | 95.5 |
| 300 | 15 | - - | - - | 98.4 |
| 300 | 60 | - - | - - | 95.5 |
| 500 | 15 | - - | - - | 97.0 |
| 500 | 60 | - - | - - | 96.3 |

**Table 4. Effectiveness of G'lutaraldehyde on infected eggs of rainbow trout and toxicity of 1, 3, and 5X concentrations to uninfected eggs at 12°C.**

| Treatment (ppm) | Exposure (min.) | Infection (%) Initial | Infection (%) Increase | Hatch (%) |
|---|---|---|---|---|
| **Efficacy** | | | | |
| (-) Control | - - | 1.2 | 20.9 | 85.3 |
| (t) Control | - - | 18.8 | 44.0 | 54.7 |
| 50 | 15 | 18.7 | 34.2 | 72.9 |
| 50 | 60 | 18.1 | 4.4 | 75.9 |
| 100 | 15 | 28.5 | 33.3 | 68.6 |
| 100 | 60 | 23.5 | 17.0 | 78.9 |
| 200 | 15 | 20.9 | 23.1 | 79.4 |
| 200 | 60 | 23.7 | 3.7 | 75.8 |
| **Toxicity** | | | | |
| (-) Control | - - | - - | - - | 94.8 |
| (t) Control | - - | - - | - - | 95.4 |
| 100 | 15 | - - | - - | 94.5 |
| 100 | 60 | - - | - - | 93.6 |
| 300 | 15 | - - | - - | 94.2 |
| 300 | 60 | - - | - - | 59.5 |
| 500 | 15 | - - | - - | 93.1 |
| 500 | 60 | - - | - - | 8.6 |

**Table 5. Effectiveness of Potassium Permanganate on infected eggs of rainbow trout and toxicity of 1, 3, and 5X concentrations to uninfected eggs at 12°C.**

| Treatment (ppm) | Exposure (min.) | Infection (%) Initial | Increase | Hatch (%) |
|---|---|---|---|---|
| **Efficacy** | | | | |
| (-) Control | - - | 2.9 | 25.8 | 81.6 |
| (t) Control | - - | 21.8 | 23.5 | 71.4 |
| 25 | 15 | 22.3 | 20.7 | 64.8 |
| 25 | 60 | 21.6 | 19.7 | 74.7 |
| 50 | 15 | 24.3 | 14.9 | 71.0 |
| 50 | 60 | 21.7 | 17.7 | 69.0 |
| 100 | 15 | 23.6 | 13.1 | 68.3 |
| 100 | 60 | 21.9 | 12.0 | 67.6 |
| **Toxicity** | | | | |
| (-) Control | - - | - - | - - | 96.8 |
| (t) Control | - - | - - | - - | 96.9 |
| 50 | 15 | - - | - - | 95.0 |
| 50 | 60 | - - | - - | 96.4 |
| 150 | 15 | - - | - - | 96.0 |
| 150 | 60 | - - | - - | 75.9 |
| 250 | 15 | - - | - - | 91.5 |
| 250 | 60 | - - | - - | 5.9 |

**Table 6. Effectiveness of VigorOx - 5% Peracetic Acid on infected eggs of rainbow trout and toxicity of 1, 3, and 5X concentrations to uninfected eggs at 12°C.**

| Treatment (ppm) | Exposure (min.) | Infection (%) Initial | Increase | Hatch (%) |
|---|---|---|---|---|
| **Efficacy** | | | | |
| (-) Control | - - | 2.4 | 24.7 | 71.8 |
| (t) Control | - - | 21.6 | 24.2 | 60.7 |
| 20 | 15 | 18.8 | 33.5 | 47.8 |
| 20 | 60 | 20.3 | 20.0 | 70.9 |
| 50 | 15 | 20.7 | 36.1 | 56.9 |
| 50 | 60 | 21.1 | 28.3 | 71.0 |
| 100 | 15 | 19.3 | 30.4 | 64.2 |
| 100 | 60 | 20.1 | 11.2 | 79.5 |
| **Toxicity** | | | | |
| (-) Control | - - | - - | - - | 97.5 |
| (t) Control | - - | - - | - - | 97.1 |
| 50 | 15 | - - | - - | 97.4 |
| 50 | 60 | - - | - - | 97.9 |
| 150 | 15 | - - | - - | 97.1 |
| 150 | 60 | - - | - - | 97.5 |
| 250 | 15 | - - | - - | 97.3 |
| 250 | 60 | | | 60.9 |

**Table 7. Effectiveness of reference and candidate fungicides on infected eggs of rainbow trout at 12°C for an exposure time of 60 minutes.**

| Chemical Name | Treatment (ppm) | Mean (N=2) infection (%) | | Mean Hatch (%) |
|---|---|---|---|---|
| | | Initial | Increase | |
| (-) Control | - - | 0.3 | **46.9** | 56.0 |
| (t) Control | - - | 27.2 | **44.9** | 19.3 |
| Malachite Green | 1.0 | 25.9 | 22.3 | 63.5 |
| Formalin | 250 | 27.5 | 39.9 | 50.9 |
| Formalin | 1,667 | 27.2 | -3.6 | 87.0 |
| Amorolfine | **100** | 29.0 | 24.1 | 67.2 |
| Glutaraldehyde | 50 | 28.4 | 23.0 | 50.3 |
| Iodine | 100 | 28.8 | 24.4 | 44.2 |
| Potassium Permanganate | 100 | 27.8 | 23.8 | 26.0 |
| Sodium Chloride | 30,000 | 28.2 | 1.8 | 78.2 |
| VigorOx | 100 | 26.8 | 38.8 | 31.0 |

**Table 8. Effectiveness of reference and candidate fungicides on infected eggs of rainbow trout at 12°C for an exposure time of 60 minutes.**

| Chemical Name | Treatment (ppm) | Mean (N=4) infection (%) Initial | Increase | Mean Hatch (%) |
|---|---|---|---|---|
| (-) Control | - - | 3.5 ± 0.424 | 62.9 ± 0.99 | 27.8 ± 3.54 |
| (t) Control | - - | 24.2 ± 2.67 | 60.0 ± 4.46 | 13.6 ± 2.66 |
| Malachite Green | 5.0 | 23.3 ± 2.51 | 0.1 ± 5.54 | 66.6 ± 2.22 |
| Formalin | 250 | 23.2 ± 1.77 | 39.0 ± 9.89 | 32.2 ± 7.41 |
| Formalin | 1,667 | 24.1 ± 1.83 | -6.0 ± 2.43 | 73.3 ± 6.15 |
| Amorolfine | 100 | 23.2 ± 2.05 | 35.4 ± 6.11 | 42.8 ± 4.28 |
| Glutaraldehyde | 200 | 23.0 ± 2.58 | 16.2 ± 8.26 | 36.3 ± 16.8 |
| Sodium Chloride | 30,000 | 23.3 ± 2.61 | 10.2 ± 5.43 | 59.5 ± 6.83 |
| VigorOx | 200 | 23.2 ± 2.12 | 37.6 ± 7.14 | 39.8 ± 9.99 |

**Table 9. Mortality in adult spring chinook salmon within 48 hours of initial treatment on** 23 June 1992.

| Chemical Name | Treatment (ppm) | Tank | Starting N | Mortality (%) |
|---|---|---|---|---|
| Glutaraldehyde | 62 | E4 | 25 | 64.0 |
| Glutaraldehyde | 62 | c2 | 22 | 50.0 |
| Glutaraldehyde | 6.2 | E6 | 23 | 0.0 |
| Glutaraldehyde | 6.2 | c3 | 23 | 0.0 |
| Formalin | 167 | E2 | 23 | 0.0 |
| Formalin | 167 | E5 | 22 | 0.0 |
| Control | - - | E3 | 22 | 0.0 |
| Control | - - | c4 | 24 | 0.0 |

Table 10. Mortality and incidence of fungal growth in adult spring chinook salmon from 26 June 1992 through 10 September 1992. Numbers indicate exposure levels of chemical treatments and the number of fish at the start of treatments, that suffered mortality, and that suffered mortality with associated fungal infection. The cumulative percentage of starting number that suffered mortality and the percentage of mortalities that had fungal infection are indicated in parentheses.

| Chemical Name | Treatment (ppm) | Tank | Starting N | Mortality N (%) | with Fungus N (%) |
|---|---|---|---|---|---|
| Formalin | 167 | E2 | 23 | 12 (52.2) | 1 (8.3) |
| Formalin | 167 | E5 | 22 | 5 (22.7) | 0 (0.0) |
| Glutaraldehyde | 6.2 | E6 | 23 | 1 (4.3) | 0 (0.0) |
| Glutaraldehyde | 6.2 | c3 | 23 | 7 (30.4) | 1 (14.3) |
| Glutaraldehyde | 31 | E3 | 22 | 1 (4.5) | 0 (0.0) |
| Glutaraldehyde | 31 | c4 | 23 | 2 (8.7) | 2 (100.0) |
| Glutaraldehyde | 15" | E4 | 8 | 3 (37.5) | 3 (100.0) |
| Glutaraldehyde | 15* | c2 | 10 | 2 (10.0) | 0 (0.0) |

* Treatment occurred once per week